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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(31) International Patent Classification ⁶ : C12N 15/12, 15/18, 15/19, C07K 14/475, 14/49, 14/50, 14/65	A1	(11) International Publication Number: WO 96/39515 (43) International Publication Date: 12 December 1996 (12.12.96)
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(21) International Application Number: PCT/US96/09001
(22) International Filing Date: 6 June 1996 (06.06.96)
(30) Priority Data:
08/465,968 6 June 1995 (06.06.95) US
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(81) Designated States: AL, AU, BG, BR, BY, CA, CN, CZ, EE,
FI, GE, IL, JP, KG, KP, KR, KZ, LT, LV, MD, MN, MX,
NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, UA, US,
UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FI,
FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: HUMAN VASCULAR ENDOTHELIAL GROWTH FACTOR 2

(57) Abstract

Disclosed is a human VEGF2 polypeptide and DNA (RNA) encoding such VEGF2 polypeptides. Also provided is a procedure for producing such polypeptide by recombinant techniques and antibodies and antagonist against such polypeptide. Also disclosed is a method of using such polypeptide for stimulating wound healing and for vascular tissue repair. Also provided are methods of using the antagonists to inhibit tumor growth, inflammation and to treat diabetic retinopathy, rheumatoid arthritis and psoriasis. Diagnostic methods for detecting mutations in the VEGF2 coding sequence and alterations in the concentration of VEGF2 protein in a sample derived from a host are also disclosed.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An isolated polynucleotide comprising a member selected from the group consisting of:
- a) a polynucleotide encoding the full length polypeptide as set forth in SEQ ID NO: 2;
 - b) a polynucleotide encoding the mature protein portion of SEQ ID NO:2;
 - c) a polynucleotide encoding the proprotein portion of SEQ ID NO: 2;
 - d) a polynucleotide encoding the human VEGF-2 polypeptide encoded by the cDNA in ATCC Deposit No. 97149;
 - e) a polynucleotide comprising the nucleotide sequence encoding a polypeptide comprising amino acid residues -46 to 373 of SEQ ID NO: 2;
 - f) a polynucleotide comprising the nucleotide sequence encoding a polypeptide comprising amino acid residues -23 to 373 of SEQ ID NO: 2;
 - g) a polynucleotide comprising the nucleotide sequence encoding a polypeptide comprising amino acids 1 to 373 of SEQ ID NO: 2;
 - h) a polynucleotide comprising the nucleotide sequence encoding a portion of the mature VEGF-2 polypeptide comprising amino acids 24 to 373 of SEQ ID NO: 2;
 - i) a polynucleotide comprising the nucleotide sequence encoding a polypeptide comprising amino acids -46 to 24 of SEQ ID NO: 2;
 - j) a polynucleotide comprising the nucleotide sequence encoding a polypeptide comprising amino acids -23 to 24 of SEQ ID NO: 2;
 - k) a polynucleotide comprising the nucleotide sequence encoding a polypeptide comprising amino acids 1 to 24 of SEQ ID NO: 2;
 - l) a polynucleotide fragment of the polynucleotide according to any one of a) to d) with the proviso that said polynucleotide fragment comprises at least 30 contiguous nucleotides of the polynucleotide of i), j) or k);
 - m) a polynucleotide fragment which hybridises to at least 30 contiguous nucleotides of the polynucleotide encoding amino acids -46 to 24 of SEQ ID NO: 2 under the following conditions: hybridisation in 0.5 M sodium peroxide



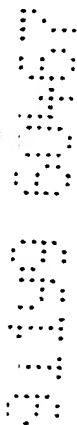
NaPO₄, 7% sodium dodecyl sulfate (SDS) at 65°C and washing with 0.5 x SSC, 0.1% SDS at 60°C or equivalent hybridisation stringency;

- n) a polynucleotide comprising the complementary form of the polynucleotide according to any one of a) to m).
2. An isolated polynucleotide according to claim 1 wherein the polynucleotide encodes the full length polypeptide as set forth in SEQ ID NO: 2.
3. An isolated polynucleotide according to claim 1 wherein the polynucleotide encodes the mature protein portion of SEQ ID NO:2.
4. An isolated polynucleotide according to claim 1 wherein the polynucleotide encodes the proprotein portion of SEQ ID NO: 2.
5. An isolated polynucleotide according to claim 1 wherein the polynucleotide encodes the human VEGF-2 polypeptide encoded by the cDNA in ATCC Deposit No. 97149.
6. An isolated polynucleotide according to claim 1 wherein the polynucleotide encodes a polypeptide comprising amino acids -46 to 373 of SEQ ID NO: 2.
7. An isolated polynucleotide according to claim 1 wherein the polynucleotide encodes a polypeptide comprising amino acids -23 to 373 of SEQ ID NO: 2.
8. An isolated polynucleotide according to claim 1 wherein the polynucleotide encodes a polypeptide comprising amino acids 1 to 373 of SEQ ID NO: 2.
9. An isolated polynucleotide according to claim 1 wherein the polynucleotide encodes a portion of the mature VEGF-2 polypeptide comprising amino acids 24 to 373 of SEQ ID NO: 2.
10. An isolated polynucleotide according to claim 1 wherein the polynucleotide encodes



a polypeptide comprising amino acids -46 to 24 of SEQ ID NO: 2.

11. An isolated polynucleotide according to claim 1 wherein the polynucleotide encodes a polypeptide comprising amino acids -23 to 24 of SEQ ID NO: 2.
12. An isolated polynucleotide according to claim 1 wherein the polynucleotide encodes a polypeptide comprising amino acids 1 to 24 of SEQ ID NO: 2.
13. An isolated polynucleotide according to claim 1 wherein the polynucleotide comprises a fragment of the polynucleotide according to any one of a) to d) with the proviso that said polynucleotide fragment comprises at least 30 contiguous nucleotides of the polynucleotides of i), j) or k).
14. An isolated polynucleotide according to claim 1 wherein the polynucleotide comprises a fragment which hybridises to at least 30 contiguous nucleotides of the polynucleotide encoding amino acids -46 to 24 of SEQ ID NO: 2 under the following conditions: hybridisation in 0.5 M sodium peroxide NaPO_4 , 7% sodium dodecyl sulfate (SDS) at 65°C and washing with 0.5 x SSC, 0.1% SDS at 60°C or equivalent hybridisation stringency.
15. An isolated polynucleotide according to claim 1 wherein the polynucleotide comprises the complementary form of the polynucleotide according to any one of claims 2 to claim 14.
16. An isolated polypeptide comprising at least 30 amino acid residues and having VEGF2 biological activity further comprising a member selected from the group consisting of:
 - a) a polypeptide comprising the amino acid sequence of the full length polypeptide of SEQ ID NO:2;
 - b) a polypeptide comprising the amino acid sequence of the mature protein of SEQ ID NO: 2;
 - c) a polypeptide comprising the amino acid sequence of the proprotein portion of



SEQ ID NO: 2;

- d) a polypeptide comprising the amino acid sequence of the mature polypeptide encoded by the cDNA contained in ATCC Deposit No. 97149;
- e) a polypeptide comprising amino acids -46 to 373 of SEQ ID NO: 2;
- f) a polypeptide comprising amino acids -23 to 373 of SEQ ID NO: 2;
- g) a polypeptide comprising amino acids 1 to 373 of SEQ ID NO: 2;
- h) a portion of the mature VEGF-2 polypeptide comprising amino acids 24 to 373 of SEQ ID NO:2;
- i) a polypeptide comprising amino acids -46 to 24 of SEQ ID NO: 2;
- j) a polypeptide comprising amino acids -23 to 24 of SEQ ID NO: 2;
- k) a polypeptide comprising amino acids 1 to 24 of SEQ ID NO: 2;
- l) a polypeptide comprising an active fragment of the VEGF2 polypeptides according to any one of a) to d) with the proviso that part of said polypeptide fragment is encoded by at least 30 contiguous nucleotides of the polynucleotide encoding the polypeptide of any one of i), j) or k);
- m) a polypeptide fragment comprising an amino acid sequence encoded by a polynucleotide sequence which hybridises to at least 30 contiguous nucleotides of the polynucleotide encoding any one of the polypeptides of i), j) or k) under the following conditions: hybridisation in 0.5 M sodium peroxide NaPO_4 , 7% sodium dodecyl sulfate (SDS) at 65°C and washing with 0.5 x SSC, 0.1% SDS at 60°C or equivalent hybridisation stringency.

- 17. An isolated polypeptide according to Claim 15 comprising the amino acid sequence of the full length polypeptide of SEQ ID NO:2.
- 18. An isolated polypeptide according to Claim 16 comprising the amino acid sequence of the mature portion of SEQ ID NO: 2.
- 19. An isolated polypeptide according to Claim 16 comprising a polypeptide comprising the amino acid sequence of the proprotein portion of SEQ ID NO: 2.



20. An isolated polypeptide according to Claim 16 comprising the amino acid sequence of the mature VEGF-2 polypeptide encoded by the cDNA contained in ATCC Deposit No. 97149.
21. An isolated polypeptide according to Claim 16 comprising amino acids -46 to 373 of SEQ ID NO: 2.
22. An isolated polypeptide according Claim 16 comprising amino acids -23 to 373 of SEQ ID NO: 2.
23. An isolated polypeptide according to Claim 16 comprising amino acids 1 to 373 of SEQ ID NO: 2.
24. An isolated portion of the mature VEGF-2 polypeptide according to Claim 16 comprising amino acids 24 to 373 of SEQ ID NO: 2.
25. An isolated polypeptide according to Claim 16 comprising amino acids -46 to 24 of SEQ ID NO: 2.
26. An isolated polypeptide according to Claim 16 comprising amino acids -23 to 24 of SEQ ID NO: 2.
27. An isolated polypeptide according to Claim 16 comprising amino acids 1 to 24 of SEQ ID NO: 2.
28. An isolated polypeptide according to Claim 16 comprising a fragment of any one of the polypeptides of Claims 16 to 20 with the proviso that part of said polypeptide fragment is encoded by at least 30 contiguous nucleotides of the polynucleotide encoding the polypeptide of any one of Claims 25 to 27.
29. An isolated polypeptide according to Claim 16 comprising an amino acid sequence



encoded by a polynucleotide sequence which hybridises to at least 30 contiguous nucleotides of the polynucleotide encoding any one of the polypeptides of Claims 25 to 27 under the following conditions: hybridisation in 0.5 M sodium peroxide NaPO_4 , 7% sodium dodecyl sulfate (SDS) at 65°C and washing with 0.5 x SSC, 0.1% SDS at 60°C or equivalent hybridisation stringency.

30. An isolated polypeptide according to any one of Claims 16 to 29 further comprising a heterologous polypeptide.
31. An isolated polypeptide according to any one of Claims 16 to 30 further comprising a homodimer.
32. An isolated polypeptide according to any one of Claims 16 to 31 wherein the polypeptide is glycosylated.
33. A composition comprising the polypeptide according to any one of Claims 16 to 32 or 40 and one or more pharmaceutically acceptable carriers and /or diluents.
34. A vector comprising the polynucleotide according to any one of Claims 1 to 15.
35. A recombinant vector comprising the polynucleotide according to any one of Claims 1-15 operatively associated with a regulatory sequence that controls gene expression.
36. A host cell comprising the polynucleotide according to any one of Claims 1-15 operably associated with a heterologous regulatory sequence or a vector comprising same.
37. The polynucleotide sequence according to any one of Claims 1-15 further comprising a heterologous polynucleotide.
38. The polynucleotide sequence of Claim 37 further comprising a polynucleotide which

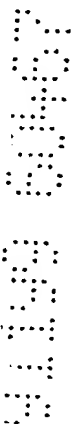


encodes a heterologous polypeptide.

39. A method for producing a VEGF-2 polypeptide at least comprising the step of culturing the genetically engineered host cell of Claim 36 for a time and under conditions suitable for the expression of the polypeptide encoded by said polynucleotide to occur.
40. A polypeptide produced by the method of Claim 39.
41. A composition comprising the polynucleotide according to any one of Claims 1-15, 37, or 38 and one or more pharmaceutically acceptable carriers and/or diluents.
42. Use of the polynucleotide according to any one of Claims 1 to 15, 37 or 38 or the polypeptide according to any one of Claims 16 to 32 or 40 in the preparation of a medicament for the treatment of a patient having need of human VEGF-2 polypeptide.
43. An antibody which is capable of binding to the polypeptide according to any one of Claims 16 to 29 with the proviso that the antibody is not capable of binding to a polypeptide consisting of amino acid residues 24 to 373 of SEQ ID NO: 2 or a fragment thereof.
44. An antisense construct capable of binding to the polynucleotide according to any one of Claims 1-15 or a complementary form thereof with the proviso that the antisense construct is not capable of binding to a polynucleotide sequence encoding amino acids 24 to 373 of SEQ ID NO: 2 or a fragment thereof.
45. A method of stimulating proliferation of endothelial cells in a patient comprising administering to the patient the polypeptide according to any one of Claims 16 to 32 or 40 for a time and under conditions sufficient for the proliferation of endothelial cells to occur.



46. The method of Claim 45 wherein the patient has vasculature tissue damage.
47. The method of Claim 45 wherein the patient has a wound, tissue damage or bone damage.
48. The method of Claim 45 wherein the patient has ischemia.
49. The method of Claim 45 wherein the patient has myocardial infarction.
50. The method of Claim 45 wherein the patient has coronary artery disease, peripheral vascular disease or CNS vascular disease.
51. The method according to any one of Claims 46 to 50 wherein the stimulation of endothelial cell proliferation is capable of further stimulating angiogenesis.
52. The isolated polynucleotide according to any one of Claims 1 to 15, 37, 38 or 44 substantially as hereinbefore described with reference to the Figures and /or Examples.
53. The isolated polypeptide according to anyone of Claims 16 to 32 or 40 substantially as hereinbefore described with reference to the Figures and/or Examples.
54. The vector of Claim 34 substantially as hereinbefore described with reference to the Figures and/or Examples.
55. The host cell of Claim 36 substantially as hereinbefore described with reference to the Figures and/or Examples.
56. The method according to any one of Claims 39, 45 to 51 substantially as hereinbefore described with reference to the Figures and/or Examples.
57. The use according to Claim 42 substantially as hereinbefore described with reference



to the Figures and/or Examples.

58. The composition of Claim 33 or 41 substantially as hereinbefore described with reference to the Figures and/or Examples.

DATED this EIGHTH day of NOVEMBER, 1999

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AUSTRALIA

Patents Act 1990

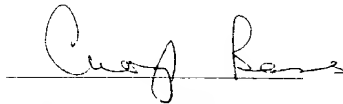
IN THE MATTER OF Australian Patent
Application Serial No. 696764 by Human
Genome Sciences, Inc.

-and-

IN THE MATTER OF Opposition thereto by
Ludwig Institute for Cancer Research

THIS IS Exhibit PAWR-2
referred to in the Statutory Declaration
of Peter Adrian Walton Rogers
made before me

DATED this 12th Day of November, 2001

A handwritten signature in cursive script, appearing to read "Craig Rane", written over a horizontal line.

(Signature of Witness)

Medical Practitioner

(12) PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. AU 199653665 B2
(10) Patent No. 726486

(54) Title
Human tumor necrosis factor delta and epsilon

(51)⁷ International Patent Classification(s)
C07H 021/02 C12N 005/00
C07H 021/04 C12N 015/09
C07K 001/00 C12N 015/63
C07K 014/00

(21) Application No: 199653665

(22) Application Date: 1996.03.14

(87) WIPO No: WO97/33902

(43) Publication Date : 1997.10.01

(43) Publication Journal Date : 1997.11.27

(44) Accepted Journal Date : 2000.11.09

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(56) Related Art
US 5487984
M.D. ADAMS ET AL. NATURE VOL.355 13/2/1992 PP 632-634